TRANSAMINASE AND PYRIDOXINE DEFICIENCY*

By STANLEY R. AMES,† P. S. SARMA, AND C. A. ELVEHJEM

(From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison)

(Received for publication, October 14, 1946)

The rôle of vitamins in enzymatic systems has been examined frequently by the determination of enzymatic activities in animals maintained on a vitamin-deficient ration. The indication that pyridoxal phosphate functions as the prosthetic group of transaminase offered an excellent opportunity to test this relationship in the case of another vitamin and to devise more stringent criteria for the deficiency method of relating vitamins and enzymes. This study of the effect of a pyridoxine deficiency on transaminase was facilitated by the use of an improved vitamin B_6-deficient ration developed in this laboratory.

While studying the reversible *in vitro* interconversion of pyridoxal and pyridoxamine, Snell (1) first suggested that vitamin B_6 was concerned in biological transamination. Schlenk and Snell (2) suggested that vitamin B_6 may function as cotransaminase, owing to the fact that the tissues of vitamin B_6-deficient rats were low in transaminase and also that pyridoxal and pyridoxamine plus adenosine triphosphate (ATP) had the ability to reactivate partially the deficient system in six out of nine trials. These conclusions and the experimental method employed have been strongly criticized (3), but the experimental basis for this criticism was later shown to be in error (4). Schlenk and Fisher (5), on the basis of isolation experiments, indicated that vitamin B_6 was involved in glutamic-aspartic transaminase. Leloir and Green (6) isolated two transaminase systems and reported that there was no vitamin B_6 present, but later Green *et al.* (7) presented evidence which points to pyridoxal phosphate as cotransaminase.

This investigation confirms and extends the findings of Schlenk and Snell (2). Observations are made concerning transaminase and succinic oxidase in normal and vitamin B_6-deficient rats, both with and without the addition of various members of the vitamin B_6 complex and their phosphates.

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. These studies were aided by a grant from the Rockefeller Foundation. Presented before the Division of Biological Chemistry of the American Chemical Society at Chicago, September, 1946.

† Present address, Research Laboratories, Distillation Products, Inc., Rochester, New York.

Methods

Albino rats, obtained from Sprague-Dawley, were placed on experiment at weaning age. One group of rats was fed the vitamin B_6-deficient ration developed by Sarma, Snell, and Elvehjem (8), and a group of control animals was fed the same diet plus 150 γ of pyridoxine hydrochloride per 100 gm. of ration. The dry weights of samples were determined by evaporating to constant weight in open crucibles in an electric oven at 110°.

Transaminase values were determined by the method of Ames and Elvehjem (9), which measures the oxalacetic acid formed in a reaction mixture containing *l*-aspartic acid, α-ketoglutaric acid, and tissue homogenate. The components of the final reaction mixture are 0.50 ml. of 0.25 M sodium potassium phosphate buffer (pH 7.3), 0.50 ml. of 0.20 M sodium aspartate, 0.50 ml. of 0.10 M sodium α-ketoglutarate[1] (one side arm), the desired amount of tissue homogenate in 0.10 M sodium phosphate buffer (pH 7.3), and a sufficient quantity of this same 0.10 M buffer to yield a total reaction volume of 2.50 ml. The transaminating reaction was allowed to proceed for exactly 10 minutes and was stopped by the addition of 0.50 ml. of aniline citrate reagent from a second side arm. The carbon dioxide evolved is a measure of the oxalacetic acid formed in this reaction. Corrections were applied for both the carbon dioxide arising because of the addition of tissue homogenate and the carbon dioxide evolution at zero tissue concentration obtained by extrapolation. In experiments in which the extent of reactivation effected by various members of the vitamin B_6 complex was determined, these solutions were added at the expense of the 0.10 M phosphate buffer.

Succinic oxidase was determined by the method of Schneider and Potter (10) and solutions to be tested for possible reactivation were added at the expense of the water.

Solutions were made up in glass-redistilled water as follows: pyridoxine monohydrochloride, Merck, 100 γ per ml.; pyridoxal monohydrochloride, Merck, 100 γ per ml.; pyridoxamine dihydrochloride, Merck, 100 γ per ml.; pyridoxal phosphate (as dibarium salt),[2] 100 γ per ml.; pyridoxamine phosphate,[3] 100 γ per ml.; ATP, 0.75 M (weighed as dibarium salt but with barium removed by precipitation as sulfate).

[1] Appreciation is expressed to Professor R. H. Burris for a generous sample of α-ketoglutaric acid.

[2] Obtained from Professor I. C. Gunsalus through the courtesy of Professor E. E. Snell. The dibarium salt was weighed out and the barium removed by precipitation with sodium sulfate. After acid hydrolysis, the solution contained 34 γ per ml. of pyridoxal, as determined microbiologically by Jesse C. Rabinowitz.

[3] Prepared from the above solution of pyridoxal phosphate by autoclaving with glutamic acid, as proposed by Snell in a private communication, on the basis of the analogous reaction which occurs with pyridoxal (1).

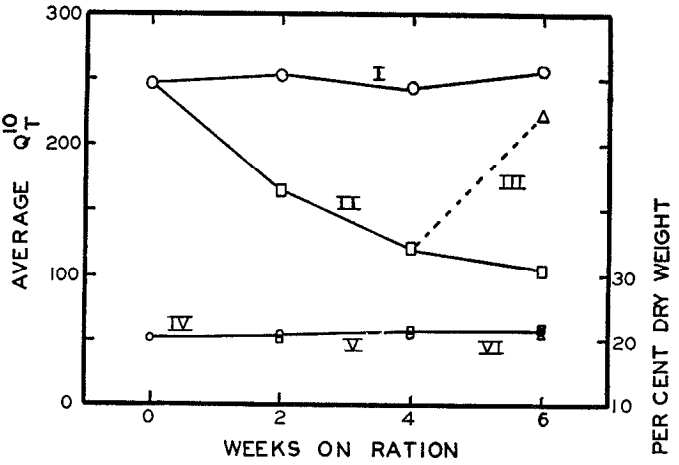

FIG. 1. Effect of vitamin B_6-deficiency on transaminase activities and dry weight determinations of heart muscle from albino rats. Curves I and IV, ○, normal; transaminase activities and dry weights respectively of tissues from rats receiving adequate vitamin B_6. Curves II and V, □, deficient; transaminase activities and dry weights respectively of tissues from rats placed at weaning age on a diet deficient in vitamin B_6. Curves III and VI, △, converted; transaminase activities and dry weights respectively of tissues from rats receiving adequate vitamin B_6 from 4 to 6 weeks after being depleted for a period of 4 weeks. Average standard deviations of the experimental determinations are Curve I, $\sigma = 16.9$; Curve II, $\sigma = 11.9$; Curve III, $\sigma = 5.3$; and Curves IV, V, and VI, $\sigma = 0.42$.

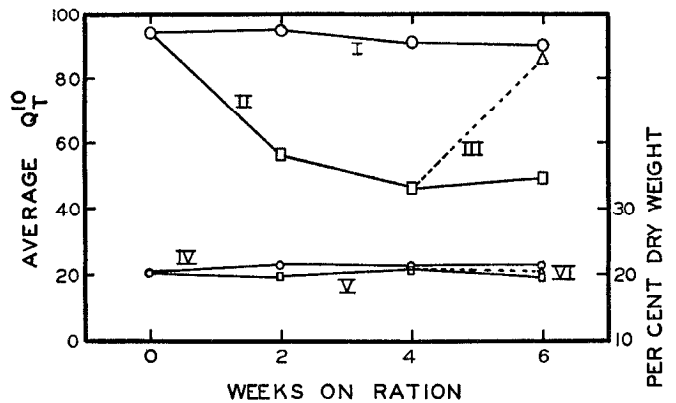

FIG. 2. Effect of vitamin B_6 deficiency on transaminase activities and dry weight determinations of kidney cortex from albino rats. Curves I and IV, ○, normal; transaminase activities and dry weights respectively of tissues from rats receiving adequate vitamin B_6. Curves II and V, □, deficient; transaminase activities and dry weights respectively of tissues from rats placed at weaning age on a diet deficient in vitamin B_6; Curves III and VI, △, converted; transaminase activities and dry weights respectively of tissues from rats receiving adequate vitamin B_6 from 4 to 6 weeks after being depleted for a period of 4 weeks. Average standard deviations of the experimental determinations are as follows: Curve I, $\sigma = 4.2$; Curve II, $\sigma = 2.5$; Curve III, $\sigma = 4.3$; and Curves IV, V, and VI, $\sigma = 0.57$.

Results

Preliminary experiments indicated that rats maintained 6 weeks on a vitamin B_6-deficient ration had low levels of transaminase in all tissues examined; *i.e.*, heart, kidney, liver, brain, and skeletal muscle. Heart muscle and kidney cortex were arbitrarily selected as the tissues to be utilized in a careful study of the effect of a vitamin B_6-deficient diet on transaminase activity.

The results obtained by determining the transaminase activity in each of the two experimental groups at 2 week intervals are indicated in Figs. 1 and 2 for heart and kidney tissue respectively. Over the 6 weeks experimental period, the animals receiving a vitamin B_6 supplement maintained their initial transaminase activity, whereas the animals on the vitamin B_6-deficient ration had transaminase activities of about 40 per cent of the con-

TABLE I

Succinic Oxidase and Pyridoxine Deficiency

Description of animals*	No. of wks. on diet	Kidney cortex		Heart muscle	
		Q_{O_2}	No. of animals	Q_{O_2}	No. of animals
Adequate pyridoxine	6	198	4	148	3
Converted†	6			143	3
Pyridoxine-deficient	6	143	4	128	4
"	4–5			138	4
"	10–12	183	2	127	4

* Albino rats were placed on indicated diets at weaning age.

† Albino rats receiving adequate pyridoxine from 4 to 6 weeks, after being previously depleted on a deficient ration for a period of 4 weeks.

trol values. The decrease in enzymatic activity is most rapid during the initial 2 week period.

One group of animals was transferred at the end of 4 weeks from the vitamin B_6-deficient ration to the ration supplemented with pyridoxine. A marked increase in transaminase activity was noted but the level of activity observed in these rats did not reach the level for those rats supplemented with pyridoxine from the beginning. These observations hold for both heart and kidney tissue.

In order to determine whether transaminase was being affected specifically by a vitamin B_6 deficiency or whether other enzymatic activities were being depressed as well, the activity of succinic oxidase was determined on both vitamin B_6-deficient rats and those receiving a pyridoxine supplement. Table I shows that the succinic oxidase activity was lower

than that in the normal animals but was not depressed nearly as much as in the case of transaminase.

The ability of various members of the vitamin B_6 complex to reactivate the depressed enzymatic activities was determined. Pyridoxal and pyridoxamine have been previously reported to be inactive, but the addition of ATP to either of the above compounds is reported partially to reactivate the system (2). The data included in Table II essentially confirm the

TABLE II

Effect of Additional Components on Transaminase and Succinic Oxidase Activities of Heart Muscle from Pyridoxine-Deficient Albino Rats

Additional component*	Q_T^{10} transaminase, normal = 250		Q_{O_2} succinic oxidase, normal = 148	
	Control	Plus component	Control average	Plus component average
Pyridoxal	146	146		
	164	164		
" + ATP	152	165	147	53†
	165	160	150	141
" phosphate	146	187		
	164	191		
	92	112		
Pyridoxamine	147	147		
" + ATP	152	130	151	57†
	165	175		
" phosphate‡	147	181	124	313§
	89	135		
	92	145		

* Additional components were added at the following levels: pyridoxal and pyridoxamine, 0.30 ml. of 100 γ per ml.; ATP, 0.20 ml. of 0.75 M; and pyridoxal phosphate and pyridoxamine phosphate, 0.50 ml. of 100 γ per ml.

† This depression of the succinic oxidase system was not observed when ATP alone was added to the reaction vessel.

‡ See footnote 3 in the text.

§ The preparation of pyridoxamine phosphate contained 10 mg. of glutamic acid per ml., which itself causes a stimulation of the succinic oxidase systems.

previous findings concerning pyridoxal and pyridoxamine, but, at the low levels added, no significant increase could be shown on the further addition of ATP. However, the addition of pyridoxal phosphate and pyridoxamine phosphate resulted in substantial increases in the transaminase activity. No stimulation was observed when tissues from control animals were used and none of the compounds tested were effective in reactivating the succinic oxidase system from either deficient or control animals.

DISCUSSION

The study of transaminase activity as a function of the age of the animal showed that in the normal animals no significant change was apparent in either heart or kidney within the 6 weeks experimental period. Transaminase in the vitamin B_6-deficient animals exhibited an initial rapid decrease followed by a plateau in the activity curve. On adding vitamin B_6 to the ration the animals showed an immediate rapid gain in transaminase activity. The albino rat apparently has only a small reserve of vitamin B_6 complex available for conversion into cotransaminase, since the enzymatic activity parallels the growth in showing rapid fluctuations when vitamin B_6 is removed or returned to the diet.

Some workers have questioned the results obtained from vitamin-deficient animals on the basis of inanition and presumed variation in water, fat, carbohydrate, and protein content of the deficient tissues. While the many secondary effects possible in a study of this type are not overlooked, it is believed that this variation has been overemphasized. In this investigation, the dry weights were carefully determined for each group and, while the dry weight varied a little from the normal in the case of kidney cortex, no variation was observed in the case of heart muscle. It is important to note that the small variations observed can by no means account for the large changes noted in the enzymatic activity. Snell, Guirard, and Williams (11) have investigated the concentrations of biotin and pantothenic acid in normal and vitamin B_6-deficient animals and observed that there were no changes in the concentration of the first two vitamins, while the concentration of vitamin B_6 was greatly depressed. It is evident from these observations that, while the tissues from vitamin-deficient animals may exhibit slight alterations in properties not directly related to the deficiency syndrome, these are of secondary importance in studying the primary effect of the vitamin deficiency. *A priori* criticisms of the vitamin-deficient animal technique for the study of enzymatic structure which are based on these secondary changes are not entirely justified.

While an enzyme depending on a particular vitamin for its activity is markedly inactivated in the vitamin-deficient animal, it has been shown that other enzymes may exhibit some depression of activity. In the attempt to circumvent these secondary effects, the vitamin or one of its derivatives could be added to the reaction mixture to determine whether any reactivation takes place. A successful experiment constitutes evidence that a relationship between the vitamin and the enzyme is probable. When the vitamin-deficient animal is used to study enzymatic structure, it is suggested that wherever possible two criteria be imposed before a relationship is postulated; first, the activity of the enzyme must show a marked

depression in the deficient animal and, on feeding the deficient animal the particular factor, the enzymatic activity must be rapidly regained; and, secondly, the addition *in vitro* of the vitamin or a derivative thereof must result in a specific reactivation.

SUMMARY

1. The heart and kidney tissues of albino rats maintained on a vitamin B_6-deficient ration exhibited about 40 per cent of the transaminase activity of similar tissues from animals receiving adequate vitamin B_6.

2. Succinic oxidase was somewhat depressed in the deficient animals, but only to 80 to 90 per cent of normal.

3. The addition of pyridoxal and pyridoxamine with or without added adenosine triphosphate was without effect at the low levels tested, but the addition of pyridoxal phosphate or pyridoxamine phosphate at approximately the same low level resulted in a substantial reactivation of the transaminase activity. None of the compounds tried had any effect in reactivating the succinic oxidase system.

4. The changes in transaminase activity parallel the changes in growth when vitamin B_6 is withheld or returned to the diet.

It is a pleasure to thank Professor E. E. Snell for his helpful advice and criticism.

BIBLIOGRAPHY

1. Snell, E. E., *J. Am. Chem. Soc.*, **67,** 194 (1945).
2. Schlenk, F., and Snell, E. E., *J. Biol. Chem.*, **157,** 425 (1945).
3. Cohen, P. P., and Lichstein, H. C., *J. Biol. Chem.*, **159,** 367 (1945).
4. Lichstein, H. C., Gunsalus, I. C., and Umbreit, W. W., *J. Biol. Chem.*, **161,** 311 (1945).
5. Schlenk, F., and Fisher, A., *Arch. Biochem.*, **8,** 337 (1945).
6. Leloir, L. F., and Green, D. E., *Federation Proc.*, **4,** 96 (1945).
7. Green, D. E., Leloir, L. F., and Nocito, V., *J. Biol. Chem.*, **161,** 559 (1945).
8. Sarma, P. S., Snell, E. E., and Elvehjem, C. A., *J. Biol. Chem.*, **165,** 55 (1946).
9. Ames, S. R., and Elvehjem, C. A., *J. Biol. Chem.*, **166,** 81 (1946).
10. Schneider, W. C., and Potter, V. R., *J. Biol. Chem.*, **149,** 217 (1943).
11. Snell, E. E., Guirard, B. M., and Williams, R. J., *J. Biol. Chem.*, **143,** 519 (1942).